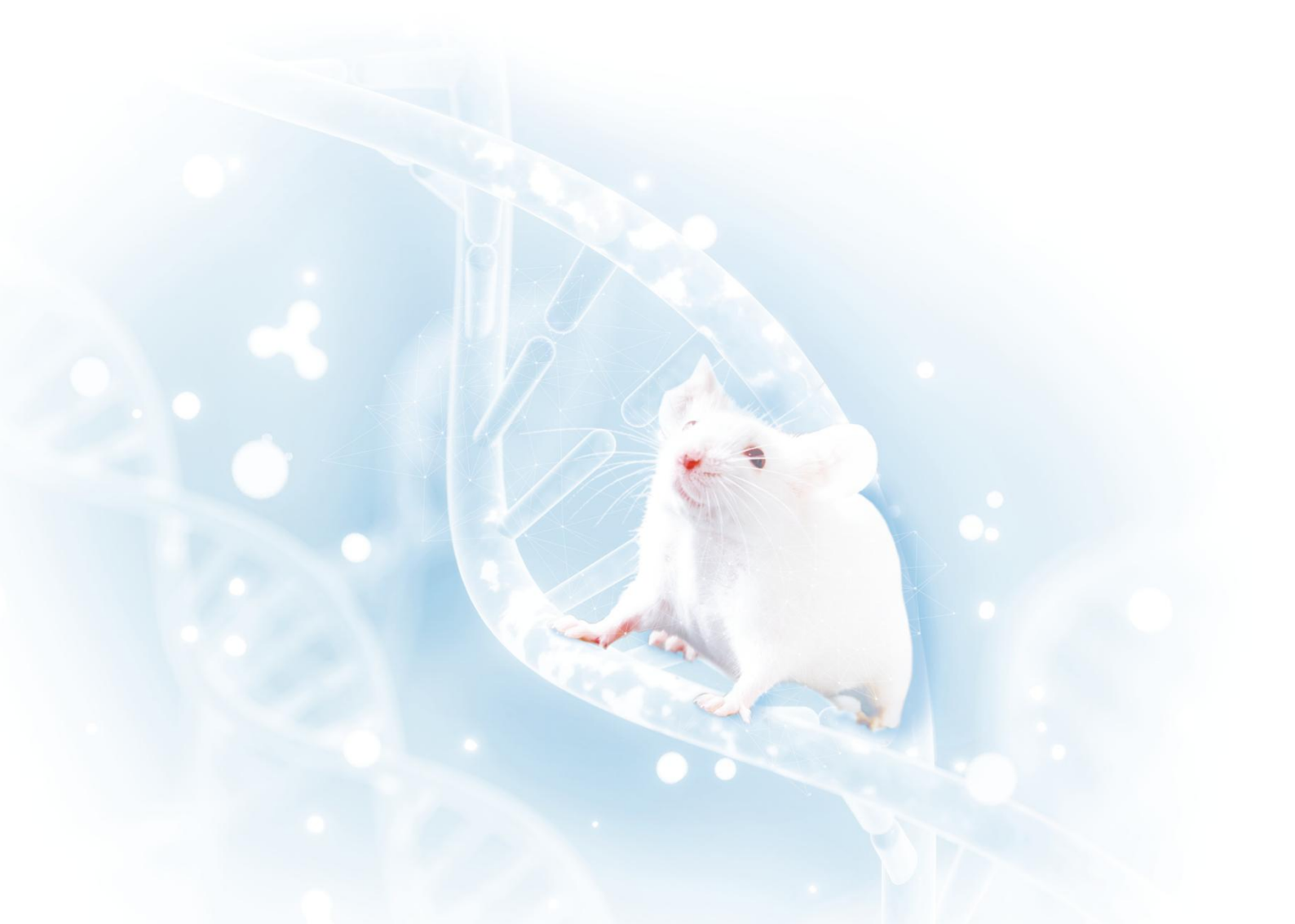
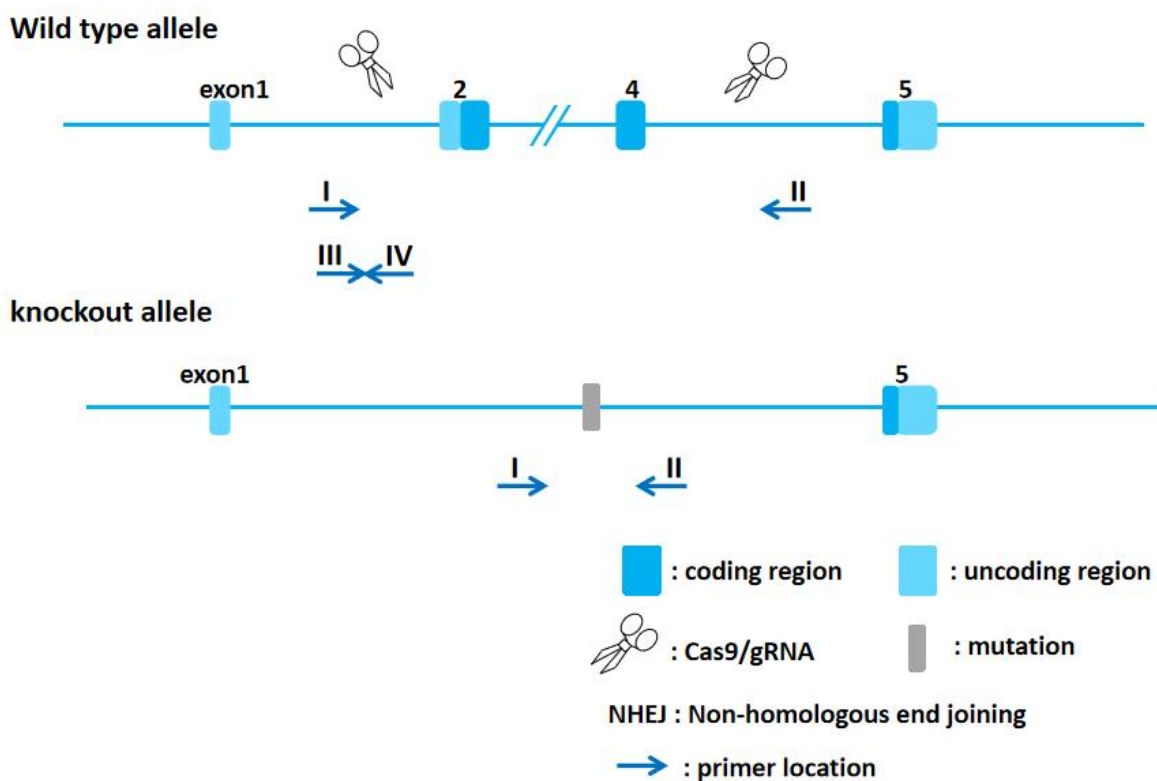


Cbx1-KO Genotyping Protocol



Common Name	Cbx1-KO	Cat. NO.	NM-KO-242165
Strain of Origin	C57BL/6J	Version	V1

Genotyping strategy



Primers

Primer	Sequence (5'→3')	Primer type
P1	ATGACCCACATGGTGCTGAC	Forward
P2	TCTAGCCGGCAATGAACAGC	Reverse
P3	TGTCGAGAATAGCGGAGTGG	Forward
P4	AGCACTTGGGAGGAGAGACA	Reverse

Expected results

Results	GeneRuler™ 1 kb DNA Ladder DL2000 0.5 µg/lane, 8 cm length gel, 1X TAE, 7 V/cm, 45 min
Genotype	Knockout type: -2925 bp Wild type: P1P2 =3632 bp; P3P4 =381 bp Heterozygote: P1P2 =3632 bp and 707 bp; P3P4 =381 bp Homozygote: P1P2 =707 bp

Note: In both wild-type and heterozygous mice, whether the P1 and P2 primers can amplify larger bands does not affect the interpretation of the results, because the purpose of designing this pair of primers is to amplify KO band

Reaction &Cycling

PCR Reaction System	Reaction Component			Volume (µl)
	ddH ₂ O			8.0
	2×Rapid Taq Master Mix			10.0
	P1(10 pmol/µl) or P3(10 pmol/µl)			0.5
	P2(10 pmol/µl) or P4(10 pmol/µl)			0.5
	Genomic DNA			1.0
	Total			20
	2×Rapid Taq Master Mix from Vazyme(Code Number: P222-01)			
Cycling Reaction	Step	Temp	Time	Note
	1	95°C	5 min	
	2	95°C	20 sec	
	3	60°C	20 sec	
	4	72°C	20 sec	35 repeats to 2
	5	72°C	5 min	
	6	12°C	Hold	